(such as IL-2, IL-7, IL-15), Th2 cell-associated cytokines, hypoxia, and estradiol (Stephen et al., 2014). Altogether, these findings underscore the relative "specificity" of the TGF-β-Foxp1 pathway.

The present work might have important clinical implications. Inhibition of TGF-β and its signaling pathway with antibodies or antisense oligonucleotides or antisense molecules targeting TGF-βRI or RII is one possible strategy for boosting anticancer immune responses. Adoptive transfer of cytotoxic T lymphocytes engineered to express a dominant-negative mutant of the TGF-β receptor is also in early development. As an alternative, the emerging, ever-more practical genome-editing technologies (such as transcription-like effector nucleases and clustered regularly interspaced short palindromic repeats) might be used to engineer T cells without TGF-β receptor subunits or downstream effectors including FOXP1 (Figure 1). Finally, the facts that FOXP1 must cooperate with other transcription factors including SMAD2 and SMAD3 and simultaneously must antagonize FOXO1 might be taken advantage of to create small molecules that disrupt specific protein-protein or protein-DNA interactions with the scope of creating a new category of checkpoint blockers.

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Plague's Partners in Crime

Kimberly M. Davis[1,2] and Ralph R. Isberg[1,2,*]
[1]Howard Hughes Medical Institute
[2]Department of Molecular Biology and Microbiology
Tufts University School of Medicine, 150 Harrison Avenue, Boston, MA 02111, USA
*Correspondence: ralph.isberg@tufts.edu
http://dx.doi.org/10.1016/j.immuni.2014.09.003

The hallmark of bubonic plague is the presence of grotesquely swollen lymph nodes, called buboes. This frenzied inflammatory response to *Yersinia pestis* is poorly understood. In this issue of *Immunity*, St. John et al. (2014) explore the mechanism by which *Y. pestis* spreads and thus leads to this striking lymphadenopathy.

In anticipation of microbial infections, the dendritic cell (DC) acts as an early immune detection system in tissue sites. Upon encounter with a microbe, DCs quickly mature and traffic to local draining lymph nodes (dLNs), where they interact with T cells and B cells to promote the development of adaptive immunity. Simultaneously, neutrophils, monocytes, macrophages, and additional phagocytic cells infiltrate tissues and attempt to eliminate the microorganism before the adaptive response is fully engaged. After clonal expansion in LNs, lymphocytes also traffic to sites of infection to combat pathogens. Receptors and signaling molecules are involved at every step along this process with the goal of orchestrating an effective immune response by multiple immune cells.

These layers of protection appear ideally suited to stop pathogens in their tracks, but could the migration of cells involved in immune defense collaborate with the pathogen to support the progress of disease? Certainly it appears that way with HIV, in which DCs carrying intact virus traffic to LNs to deliver the microbe to a site rich in susceptible host cells. Bacterial pathogens that preferentially replicate within lymphoid tissue, such as *Salmonella* and *Yersinia* species, could also follow this model, but the cellular basis for their migration into these tissues is poorly understood (Viboud and Bliska, 2005; Zhang et al., 2008a; Zhang et al., 2008b). In this issue of *Immunity*, St. John et al. (2014) show that DCs play an important role in delivering *Yersinia pestis* into regional LNs, whereas other phagocytic cells promote inter-LN spread and thus support disease progression of *Y. pestis*.

The terrifying bubonic plague, responsible for the elimination of large portions of the European population during epidemic outbreaks, is a consequence of *Y. pestis* inoculation by infected fleas. An impressive sign of disease is the bubo, which is the result of an apparent unrestrained swelling of LNs proximal to the

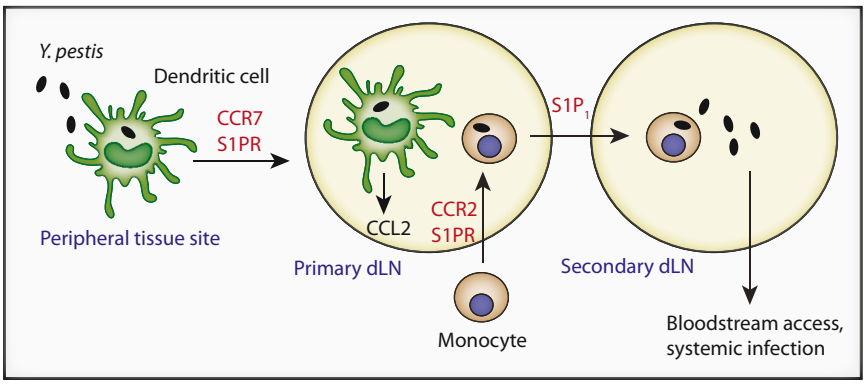

Figure 1. After Inoculation, Infected DCs Are Recruited from the Periphery to Primary LNs via CCR7 and S1PRs, where They Produce CCL2 and Recruit Monocytes via CCR2 and S1PRs to Primary dLNs

Infected monocytes transport bacteria intracellularly to secondary LNs, where they begin to replicate extracellularly and spread to the bloodstream and tissue sites. Trafficking of infected monocytes (CX_3CR1^+) between primary and secondary LNs via the receptor $S1P_1$ is particularly important in disease progression.

fleabite. Dissemination from the swollen node to additional tissue sites can lead to other forms of disease, including pneumonic plague, which is highly contagious and usually lethal in the absence of antimicrobial therapy. Little is known about what occurs between inoculation and bubo formation, in part because *Y. pestis* disease typically progresses so quickly that it is difficult to assess early events. For this reason, the study by St. John et al. (2014) is something of a breakthrough and gives us insight into how the relatively innocuous bite of a flea can lead the pathogen to target regional LNs and cause dramatic swelling in the targeted organ.

The authors used an attenuated strain of *Y. pestis* defective in iron acquisition, which slows bacterial replication in iron-depleted tissues, allowing the workers to examine events that would normally happen too quickly for visualization. In this model, bubo formation occurred by 24 hr, and immune cells harboring intracellular bacteria could be visualized within dLNs, similar to what is typically described for clinical infections (Butler, 1994). Using bacteria expressing orange fluorescent protein, they found a temporal separation in the targeting of host cells by bacteria: DCs represented the niche assumed by bacteria at early time points by delivering bacteria into dLNs, and a subsequent temporally distinct shift to monocyte and macrophage association drove movement between LNs. DCs were recruited to dLNs via the chemokine receptor CCR7, and DC recruitment was necessary for CCL2 production and subsequent monocyte recruitment, indicating that DCs play two roles in this process. First, they support initial bubo formation by causing bacteria to target dLNs. Second, their chemokine production recruits cell types that support the spread of bacteria throughout the lymphatic system (Figure 1). The absence of CCR7 significantly improved disease outcome by lowering bacterial replication and spread to other tissue sites. In comparison, there was only minor attenuation in *Ccr2*$^{-/-}$ mice, indicating that the ability of DCs to recruit macrophages and monocytes is most likely secondary to their primary role in supporting bacterial transport to dLNs. The caveat to these experiments, of course, is that all assays involved the slow-growing mutant, and it remains unclear whether fully virulent strains also require DCs for spread. In addition, the infectious doses used in these experiments are many orders of magnitude larger than what is typically analyzed in the mouse model. In spite of these technical issues, the results are indeed remarkable and connect disease processes promoted by bacterial pathogens to those normally associated with viral diseases, such as HIV.

The spread of *Y. pestis* from LNs to other sites, as occurs in pneumonic plague, requires that the microorganism break through the cellular response and move systemically. This could result from dLN tissue damage leading to bloodstream access and systemic spread. In this mouse model, the vasculature surrounding the dLN appeared intact despite excessive inflammation, suggesting that there might be a cellular route for spread. Phagocyte populations have been isolated throughout the lymphatic vasculature (Bell, 1979), indicating that they have the ability to move from primary dLNs and potentially spread microbes. The authors show in this model that *Y. pestis* hijacks this internodal phagocyte trafficking pathway as well to directly deliver intracellular bacteria to secondary LNs and promote spread. There was evidence of extracellular growth of bacteria within the secondary LNs, which could promote passive movement of bacteria through LNs to the bloodstream (Figure 1).

T and B cells act as sentinel cells within secondary lymphoid organs, such as the spleen, LNs, and Peyer's patches, by constantly surveying the lymphatic system for antigen presentation by phagocytic cells. Lymphocyte egress from these organs is primarily regulated by sensing of extracellular sphingosine-1-phosphate (S1P), which binds S1P receptors (S1PRs) (Cyster and Schwab, 2012). S1PRs come in five isoforms and are differentially expressed on various cell types, including phagocytes, arguing that these receptors are important for inter-LN movement by phagocytes. DC migration to and from LNs requires the S1PRs $S1P_3$ and/or $S1P_1$ (Rathinasamy et al., 2010), although there is little information on the roles of S1PRs in the migration of other phagocyte populations.

To address the role of S1PRs in phagocyte egress from the LNs, St. John et al. used the pan S1PR inhibitor FTY720 to show that S1PRs play an important role in the migration of infected phagocytes from the periphery to primary dLNs, as well as in inter-LN traffic. This indicated that DC migration to dLNs occurs through multiple pathways, two of which are sensed by CCR7 and S1PRs. The importance of S1PR-dependent mononuclear cell migration in mediating bacterial spread was emphasized in this work by the fact that elimination of $S1P_1$ expression, specifically within mononuclear cells, interfered with disease progression (Figure 1).

The results presented in this manuscript indicate that DCs play a dual role in collaborating with *Y. pestis* to promote lymphadenopathy and disease spread:

they transport bacteria from tissue sites to initiate bubo formation and, via the production of chemokines, recruit monocytes that allow the spread of bacteria to secondary LNs. However, these two roles for DCs might not be unique to this cell type. Monocyte subsets can also traffic to LNs, raising the possibility that they could similarly initiate entry from tissues into dLNs (Jakubzick et al., 2013). The authors' experiments with an $S1P_1$ inhibitor argue that internodal trafficking is most likely the main contribution of monocytes to disease progression, thus providing a simple two-cell model of DC-mediated initial dLN entry followed by monocyte-mediated secondary LN spread.

The ability to block migration of host cells, either from the periphery to dLNs or between LNs, could be an important therapeutic result. In this study, mice were treated with FTY720 prior to and during infection with *Y. pestis*, and it would be interesting to see whether administration following inoculation could also block LN egress. Spread to secondary LNs occurred within 24 hr postinoculation in the model of St. John et al., so it remains unclear whether it is feasible to apply this treatment regimen to prevent disease progression. It is also unclear whether host-cell-dependent egress continued after 24 hr postinoculation because many bacteria were found extracellularly at later time points. Even so, if pharmacological inhibitors can block the spread of the disease within the host, they could be powerful adjuvants used in combination with antimicrobials to limit spread and slow bacterial growth. This mouse model, utilizing an attenuated strain that affects the kinetics of disease progression, will most likely provide a platform for further experiments to address the efficacy of therapeutic treatment options.

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Gut Microbiota: A Natural Adjuvant for Vaccination

Oliver Pabst[1,*] and Mathias Hornef[2,3]
[1]Institute of Molecular Medicine, RWTH University, 52074 Aachen, Germany
[2]Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, 30625 Hannover
[3]Institute of Medical Microbiology, RWTH University, 52074 Aachen, Germany
*Correspondence: opabst@ukaachen.de
http://dx.doi.org/10.1016/j.immuni.2014.09.002

In this issue of *Immunity*, Oh et al. (2014) reveal an unappreciated facet of how the microbiota influences immune responses. Immunity to nonadjuvanted vaccines depends on Toll-like-receptor-5-mediated sensing of the microbiota.

The distal gut is colonized with an astonishing 10^{12} bacteria per gram of gut content. Other body surfaces are also colonized by microbes, which are collectively referred to as the microbiota. The term "supraorganism" has been coined to describe the fact that we are "running fermenters," carrying numerically more bacteria than we have cells in our body. However, we are only beginning to understand the impact of the microbiota on health.

Perhaps not surprisingly, the microbiota constantly produce and release potent immunostimulatory molecules, which significantly affect the immune system. Thus, germ-free animals, i.e., animals bred in the absence of any viable microbes, show substantial differences in their intestinal mucosal immune system; such differences include underdeveloped Peyer's patches, very few plasma cells, and reduced numbers of T cells. An exciting new development comes with the emerging mechanistic understanding of the influence of the microbiota on the host's immune system. Strikingly, effects are not restricted to the colonized mucosal tissue but are also observed at systemic body sites. For example, the gut microbiota has been shown to affect neutrophil maturation in the bone marrow, susceptibility to type 1 diabetes, and experimental encephalomyelitis. In this issue of *Immunity*, Oh et al. (2014) show that the gut microbiota can impact vaccination to flu.

Two seasonally administrated flu vaccines are available in the USA: a nonadjuvanted subunit vaccine (trivalent